

5

[illegible]

•

1. A method for encapsidating a recombinant poliovirus nucleic acid, comprising the steps of:

5 (a) providing a recombinant poliovirus nucleic acid which lacks the entire P1 capsid precursor region of the poliovirus genome and an expression vector lacking an infectious poliovirus genome, the nucleic acid of which encodes poliovirus P1 capsid precursor protein and directs expression of the poliovirus P1 capsid precursor protein;

10 (b) contacting a host cell with the recombinant poliovirus nucleic acid and the expression vector under conditions appropriate for introduction of the recombinant poliovirus nucleic acid and the expression vector into the host cell; and

15 (c) obtaining a yield of encapsidated viruses which substantially comprises encapsidated recombinant poliovirus nucleic acid.

2. The method of claim 1 wherein the expression vector is introduced into the host cell prior to the introduction of the recombinant poliovirus nucleic acid.

20 3. The method of claim 1 wherein the recombinant poliovirus nucleic acid is derived from a poliovirus serotype selected from the group consisting of poliovirus type I, poliovirus type II, and poliovirus type III.

25 4. The method of claim 1 wherein the nucleotide sequence of the recombinant poliovirus nucleic acid which encodes the P1 capsid precursor protein is replaced by a foreign nucleotide sequence encoding, in an expressible form, a foreign protein or fragment thereof.

5. The method of claim 1 wherein the expression vector comprises a virus.

30 6. The method of claim 5 wherein the virus is a recombinant vaccinia virus.

7. The method of claim 6 wherein the nucleic acid of the recombinant vaccinia virus encodes the poliovirus P1 capsid precursor protein and directs expression of a nucleotide sequence encoding the poliovirus P1 capsid precursor protein.

35 8. The method of claim 1 wherein the expression vector comprises a plasmid.

9. The method of claim 4 wherein the foreign nucleotide sequence is selected from the group consisting of the *gag* gene, the *pol* gene, and the *env* gene of human immunodeficiency virus type 1.

5 10. The method of claim 9 wherein the foreign nucleotide sequence is the *gag* gene of human immunodeficiency virus type 1.

11. The method of claim 10 further comprising a nucleotide sequence encoding at least two amino acids at the C-terminus of the *gag* protein of human immunodeficiency virus  
10 type 1 which comprise a cleavage site for poliovirus 2A protease.

12. The method of claim 11 wherein the nucleotide sequence encodes the following amino acids at the C-terminus of the *gag* protein of human immunodeficiency virus type 1:

15

Thr-Lys-Asp-Leu-Thr-Thr-Tyr-Gly (SEQ ID NO: 15)

13. The method of claim 4 wherein the foreign nucleotide sequence is a gene which encodes a human tumor-associated antigen.

20

14. The method of claim 13 wherein the human tumor-associated antigen is carcinoembryonic antigen.

15. The method of claim 14 wherein the gene encoding carcinoembryonic antigen  
25 does not encode a signal sequence.

16. The method of claim 15 further comprising a nucleotide sequence encoding at least two amino acids at the C-terminus of the carcinoembryonic antigen which comprise a  
30 cleavage site for poliovirus 2A protease.

17. The method of claim 16 wherein the nucleotide sequence encodes the following amino acids at the C-terminus of the carcinoembryonic antigen:

35 Thr-Lys-Asp-Leu-Thr-Thr-Tyr-Gly (SEQ ID NO: 15)

18. The method of claim 1 wherein the host cell is a mammalian host cell.

19. A method for encapsidating a recombinant poliovirus nucleic acid, comprising the steps of:

5 (a) providing a recombinant poliovirus nucleic acid which lacks the entire P1 capsid precursor region of the poliovirus genome and a recombinant vaccinia virus, the nucleic acid of which encodes poliovirus P1 capsid precursor protein and directs expression of the poliovirus P1 capsid precursor protein; and

10 (b) contacting a mammalian host cell with the recombinant poliovirus nucleic acid and the recombinant vaccinia virus under conditions appropriate for introduction of the recombinant poliovirus nucleic acid and the recombinant vaccinia virus into the mammalian host cell; and

15 (c) obtaining a yield of encapsidated viruses which substantially comprises encapsidated recombinant poliovirus nucleic acid.

20 20. The method of claim 19 wherein the nucleotide sequence of the recombinant poliovirus nucleic acid which encodes the P1 capsid precursor protein is replaced by a foreign nucleotide sequence encoding, in an expressible form, a foreign protein or fragment thereof.

21. The method of claim 20 wherein the foreign nucleotide sequence is selected from the group consisting of the *gag* gene, the *pol* gene, and the *env* gene of human immunodeficiency virus type 1.

25 22. The method of claim 22 wherein the foreign nucleotide sequence is the *gag* gene of human immunodeficiency virus type 1.

30 23. An encapsidated recombinant poliovirus nucleic acid produced by the method of claim 1.

24. An encapsidated recombinant poliovirus nucleic acid produced by the method of claim 19.

35 25. A recombinant poliovirus nucleic acid which lacks the entire P1 capsid precursor region of the poliovirus genome.

26. The recombinant poliovirus nucleic acid of claim 25 which is encapsidated.

27. An immunogenic composition, comprising:

an encapsidated recombinant poliovirus nucleic acid in which a foreign nucleotide sequence has been substituted for the entire P1 capsid precursor region of the poliovirus genome, the foreign nucleotide sequence encoding, in an expressible form, an immunogenic protein or fragment thereof; and

5 a physiologically acceptable carrier.

28. The composition of claim 27 wherein the immunogenic protein or fragment thereof is a human immunodeficiency virus type 1 protein or fragment thereof.

10 29. The composition of claim 28 wherein the human immunodeficiency virus type 1 protein is selected from the group consisting of the human immunodeficiency virus type 1 *gag* protein, the human immunodeficiency virus type 1 *pol* protein, and the human immunodeficiency virus type 1 *env* protein.

15 30. The composition of claim 29 wherein the human immunodeficiency virus type 1 protein or fragment thereof comprises the human immunodeficiency virus type 1 *gag* protein (SEQ ID NO: 17).

20 31. The composition of claim 27 wherein the immunogenic protein or fragment thereof is a human tumor-associated antigen or fragment thereof.

32. The composition of claim 31 wherein the human tumor-associated antigen is carcinoembryonic antigen.

25 33. An immunogenic composition, comprising:  
a recombinant poliovirus nucleic acid having the nucleotide sequence encoding, in an expressible form, the *gag* protein of human immunodeficiency virus type 1 substituted for the entire P1 capsid precursor region of the poliovirus genome; and  
a physiologically acceptable carrier.

30 34. The composition of claim 33 wherein the recombinant poliovirus nucleic acid is encapsidated.

35 35. A method for stimulating an immune response to an immunogenic protein or fragment thereof, in a subject, comprising  
administering, in a physiologically acceptable carrier, an effective amount of a composition comprising a recombinant poliovirus nucleic acid having a foreign nucleotide sequence encoding, in an expressible form, an immunogenic protein or fragment thereof substituted for the entire P1 capsid precursor region of the poliovirus genome.

36. The method of claim 35 wherein the recombinant poliovirus nucleic acid is encapsidated.

5 37. The method of claim 35 wherein the composition is administered orally or by intramuscular injections.

38. The method of claim 35 wherein the immunogenic protein or fragment thereof is a human immunodeficiency virus type 1 protein or fragment thereof.

10 39. The method of claim 38 wherein the human immunodeficiency virus type 1 protein or fragment thereof is selected from the group consisting of the *gag* protein, the *pol* protein, and the *env* protein of human immunodeficiency virus type 1.

15 40. The method of claim 39 wherein the human immunodeficiency virus type 1 protein or fragment thereof comprises the human immunodeficiency virus type 1 *gag* protein (SEQ ID NO: 17).

20 41. The method of claim 35 wherein the immunogenic protein or fragment thereof is a tumor-associated antigen or fragment thereof.

42. The method of claim 41 wherein the tumor-associated antigen is carcinoembryonic antigen.

25 43. A method for stimulating in a subject an immune response to the *gag* protein of the human immunodeficiency virus type 1, comprising  
administering, in a physiologically acceptable carrier, an effective amount of a composition comprising an encapsidated recombinant poliovirus nucleic acid having the nucleotide sequence of the human immunodeficiency virus type 1 *gag* gene, in expressible  
30 form, substituted for the entire P1 capsid precursor region of the poliovirus genome.

44. A method for stimulating in a subject an immune response to carcinoembryonic antigen, comprising  
administering, in a physiologically acceptable carrier, an effective amount of a  
35 composition comprising an encapsidated recombinant poliovirus nucleic acid having the nucleotide sequence of the gene encoding the carcinoembryonic antigen, in expressible form, substituted for the entire P1 capsid precursor region of the poliovirus genome.

45. A method for stimulating an immune response to a foreign protein, or fragment thereof, in a subject, comprising the steps of:

(a) removing host cells from the subject; and

(b) contacting the host cells with

(i) a recombinant poliovirus nucleic acid having a foreign nucleotide sequence substituted for the entire P1 capsid precursor region of the poliovirus genome; and

(ii) an expression vector lacking an infectious poliovirus genome, the nucleic acid of which encodes poliovirus P1 capsid precursor protein and directs expression of the P1 capsid precursor protein; and

(c) maintaining the cultured host cells under conditions appropriate for introduction of the recombinant poliovirus nucleic acid and the expression vector into the host cells, thereby generating modified host cells which express a foreign protein or fragment thereof encoded by the foreign nucleotide sequence; and

(d) reintroducing the modified host cells into the subject.